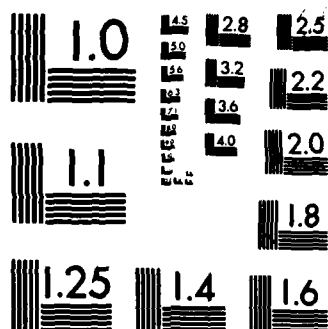MICROCOPY RESOLUTION TEST CHART

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The Mechanism of Anaerobic (Microbial) Corrosion

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U.S. DEPARTMENT OF COMMERCE
National Bureau of Standards
National Measurement Laboratory
Center for Materials Science
Inorganic Materials Division
Chemical and Biodegradation Processes Group
Washington, DC 20234

June 1983

Technical Summary Report Number 1
For the Period June 1, 1982 - December 31, 1982

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THE MECHANISM OF ANAEROBIC (MICROBIAL) CORROSION

W. P Iverson and G. J. Olson

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Washington, DC 20234

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Bacterial	Sulfate-reducing bacteria	
Anaerobic	Hypophosphite reduction	
Mechanism	Phosphine	
Iron phosphide	Volatile phosphorus component	

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

This report in the form of three papers describes research into the role of bacteria in anaerobic corrosion processes. During the year we have given more evidence for a novel mechanism of anaerobic corrosion in which a volatile, highly reactive phosphorous compound is produced as a result of the activities of sulfate-reducing bacteria (Desulfovibrio desulfuricans).

The corrosion product is an amorphous type of iron phosphide which can be detected by the formation of phosphine upon its acidification. Phosphine (in

addition to H_2S) has been detected from all the cases of suspected anaerobic corrosion (including tubercles from the inside of water pipes) examined so far.

In examining the headspace over growing cultures of <u>Desulfovibrio</u> to detect this volatile phosphorus containing compound, using a gas chromatograph (GC) with a flame photometric detector (FPD) specific for phosphorus and sulfur, two sulfur compounds, in addition to H_2S, were detected and identified. These compounds, methylmercaptan, and dimethyldisulfide, were found to be relatively non-corrosive to iron under anaerobic conditions. No volatile phosphorus compounds were detected.

In postulating that the phosphorus compound might be an intermediate of hypophosphite reduction (viz. PH_3O or PH_2OH) attempts were made to reduce the hypophosphite using the immediate corrosion of mild steel (immediate blackening of the medium) as an indicator. It was eventually found that hydrogen sulfide reacts with hypophosphite (as well as phosphate and phosphite) to form, in the presence of mild steel, iron phosphide, simulating the corrosion of iron by <u>Desulfovibrio</u>.

The compound appears too reactive to be detected by the GC-FPD although its presence can be detected: (1) by absorbing the compound in acidified permanganate solution and detecting phosphorus by the phosphomolybdate test and (2) by causing highly rapid corrosion of iron under anaerobic conditions to form iron phosphide. Confined in a hydrogen atmosphere at room ambient temperature, phosphine has been detected as a possible degradation product after several weeks. Characterization of the compound by mass spectrometry is in progress.

It remains to be determined whether anaerobic corrosion is caused by the formation of the compound inside the bacterial cell or outside the cell through the action of hydrogen sulfide on phosphorus-oxygen compounds. It is suspected that both modes of formation probably occur.

The Mechanism of Anaerobic (Microbial) Corrosion

Technical Summary Report Number 1 for the Period
June 1, 1982 to December 31, 1982

W. P. Iverson and G. J. Olson

This is the first technical summary report of a four-year project, whose objective is to determine the mechanism of anaerobic, microbial corrosion of iron by sulfate-reducing bacteria and evaluate prospective methods for inhibiting this type of corrosion.

(1) Development of a diagnostic procedure for anaerobic corrosion products employing a gas chromatograph with a flame photometric detector. Examination of the volatile species from acidified corrosion products has indicated the presence of phosphine (from iron phosphide) in addition to hydrogen sulfide.

(2) Through use of this technique iron phosphide has been established as an anaerobic corrosion product in the field and laboratory.

(3) Demonstrated that the anaerobic corrosion product, iron phosphide, is produced by the action of a highly reactive volatile phosphorus containing compound produced by sulfate-reducing bacteria (Desulfovibrio desulfuricans).

(4) Demonstrated that a highly reactive phosphorus compound is produced by the action of hydrogen sulfide on phosphate, phosphite and hypophosphite. Phosphine is formed as a degradation compound after a period of time (one to two weeks).

(5) Demonstrated that this abiotically produced phosphorus compound (hydrogen sulfide plus hypophosphite) also reacts with bulk iron to form iron phosphide.

(6) Demonstrated the formation of the relative non-corrosive compound methylmercaptan and dimethyldisulfide by sulfate-reducing organism.

This report describes the details of the above accomplishments by collecting together a preprint of a paper given at the National Association of Corrosion Engineers, 1983 Annual Meeting and a manuscript of a paper that was given at a Corrosion Conference at the National Physical Laboratory, Teddington, England (March 8-10, 1983) and is in the process of publication.

CORROSION 83

ANAEROBIC CORROSION MECHANISMS

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ABSTRACT

A discussion of the mechanisms which have been proposed to account
for the anaerobic corrosion of iron by the action of sulfate-
reducing bacteria primarily within the genus Desulfovibrio.
Proposed mechanisms have ranged from the postulated: (a) direct
bacterial removal of hydrogen (electrons) from the surface of iron;
(b) hydrogen removal by iron sulfide; (c) bacterial removal of
hydrogen from iron sulfide, to (d) the liberation of a corrosive
metabolite by the bacteria.

INTRODUCTION

When first discovered in Holland, the corrosion of iron in the absence
of oxygen was quite astounding, as corrosion had always been associated
with the presence of oxygen. Shortly after this type of corrosion, called
anaerobic corrosion, was discovered, von Wolzogen Kühr and van der Vlugt
associated this type of corrosion with the presence of the sulfate-
reducing bacterium, Desulfovibrio desulfuricans (1). At about this time
Stephenson and Stickland (2) had demonstrated that this bacterium was able
to utilize molecular hydrogen for the reduction of sulfate to hydrogen
sulfide. Using this fact, von Wolzogen Kühr and van der Vlugt (1) proposed
a cathodic depolarization theory to account for the corrosion of iron by
these bacteria. Since that time, evidence for and against this theory has
mounted and a few new theories have been proposed. This paper will briefly

review the evidence for and against the cathodic depolarization theory
as well as discuss a proposed new mechanism. These mechanisms must account
for corrosion in the absence of oxygen and at a neutral or near neutral pH.
I will exclude the effects of oxygen concentration cells in which
sulfate-reducing bacteria probably do play a role, but in which oxygen
may also be involved.

CATHODIC DEPOLARIZATION THEORY

A. Bacterial cell hydrogenase as a depolarizing agent.

An outline of the theory as originally proposed by von Wolzogen Kühr
and van der Vlugt (1) is shown in Fig. 1. The essential portion of this
theory is the proposed removal of hydrogen from the surface of the iron
by the bacterium, Desulfovibrio desulfuricans (Step IV) at a cathodic
area and causing the iron to go into solution as ferrous ions (Step II).
The secondary reactions include the reaction of the Fe^{++} ions with the
sulfide and hydroxyl ions as in Steps V and VI, respectively.

Most of the literature on microbial corrosion has been concerned with
evidence for and against this theory and has been well reviewed in the
last several years by Miller and King (3), Miller (4), Davis (5),
Iverson (6), Costello (7), and Trapathi (8).

In earlier studies by Booth and Tiller (9), Tiller and Booth (10),
a direct relationship was found between the hydrogenase activity (the
enzyme employed in hydrogen uptake), the cathodic depolarization activity
(as measured by cathodic polarization measurements) and the weight loss
of mild steel coupons, Booth and Wormwell (11). These studies were made
using batch cultures of sulfate-reducing bacteria.

B. Iron sulfide as a depolarizer.

In later work, however, using semicontinuous and continuous cultures,
Booth et al. (12) did not find this direct correlation between the
hydrogenase activity and the corrosion rate. Using high concentrations
of ferrous ions in the medium, Booth et al. (13) found that both hydrogenase-
positive and -negative strains produced quite high corrosion rates of
the order of 1.023 mm/y (220 mdd). Since no film formation was found to
occur, it was postulated by Booth et al. (14) that iron sulfide itself
caused the cathodic depolarization.

This stimulation of the corrosion rates by the presence of additional
iron had also been observed much earlier by Adams and Farrer (15).
Mara and Williams (16) believed that the bulk iron sulfide was involved,
acting as a cathode and absorbing hydrogen in proportion to the cationic
defects in the iron sulfide.

C. Iron sulfide plus bacterial hydrogenase as a depolarizer.

King et al. (17) reported that the iron sulfide was more corrosive than
had been predicted by this mechanism and had earlier postulated (18)
that the bacteria on the surface of the ferrous sulfide continually

"regenerated" or depolarized the iron sulfide by removal of atomic hydrogen as a result of their hydrogenase activity.

An additional possibility, suggested by Miller (4), was that the newly formed iron sulfide, as a result of its physical movement by the bacterial cells, caused fresh iron sulfide surfaces to be constantly brought into contact with the steel.

D. Hydrogen sulfide as a depolarizer.

In contrast to the above postulated mechanism, Costello (19) proposed that the cathodic depolarizing activity of the sulfate-reducing bacteria was due to the cathodic activity of the hydrogen sulfide produced by these organisms.

E. Model of cathodic depolarization (hydrogenase).

Using a system employing coupons on the surface of agar, Iverson (20) demonstrated the cathodic depolarization effect with a redox dye, but not with sulfate as an electron acceptor. He placed mild steel (1010) coupons in contact with masses of hydrogenase-positive sulfate-reducing bacteria on an agar surface containing benzyl viologen as an electron acceptor (in place of sulfate) and electrically connected to coupons (via a microammeter) not in contact with the bacterial cells. After several (ca. 17 hrs) hours in an inert atmosphere, it was observed that the benzyl-viologen under the coupon, in contact with the cells, was reduced and that iron went into solution under the coupon not in contact with the cells. A sustained current (1 uA/cm^2) could be measured (Fig. 2). When sulfate was substituted for benzyl viologen in the agar, no such cathodic depolarization effect could be noted and no current could be measured. Blackening was, however, found to occur in the agar under the coupon in contact with the bacterial cells. Blackening was also found in the agar containing yeast extract without any added electron acceptor.

CORROSIVE METABOLITE THEORY

In contrast to the proposed theories for cathodic depolarization by bacteria (hydrogenase), by iron sulfide, by iron sulfide plus bacteria (hydrogenase), or by hydrogen sulfide, evidence was presented by Iverson (6)(21) which indicated that the primary cause of bacterially produced anaerobic corrosion was due to a highly corrosive metabolic product produced by sulfate-reducing bacteria. For corrosion to occur by this mechanism, the corrosive metabolite must have access to the bare surface of the iron.

In studies of cathodic depolarization, using coupons placed on the surface of agar, as previously discussed, Iverson (22) observed that blackening in the agar was found to occur under a coupon in contact with the cells in the absence of any electron acceptor in the agar. Subsequently Iverson (22) observed blackening (within eight hours) of a one percent sterile yeast extract solution under a hydrogen atmosphere containing a mild steel coupon and a suspension of cells of the API strain of Desulfovibrio desulfuricans. Upon removal of the precipitate by centrifugation and

introduction of a new steel coupon in the supernatent, blackening of the solution was again observed under a hydrogen atmosphere. This was repeated a third time. The black centrifugates from the three steel exposures were collected and dried under vacuum. X-ray powder pattern analysis of the product (originally amorphous) heated in a vacuum oven to 1232 °C and allowed to cool for 24 hours indicated the presence of iron phosphide (Fe_2P). These observations suggested that a phosphorous containing metabolite was formed. In this connection, it should be mentioned that hydrogen sulfide reacts with iron at neutral or near neutral pH values to form a black film of iron sulfide on the surface of the iron and the surrounding medium remains free of any black precipitates. Ewing (23) has indicated that hydrogen sulfide does, however, blacken a surrounding aqueous medium containing metallic iron at low pH values.

In electrochemical studies to measure the corrosion rate of mild steel in an aged sea water medium containing casein and soybean digests, using a marine strain of Desulfovibrio, Iverson (24) found that the corrosion current density decreased to a very low value (0.1 to 0.5 $\mu A/cm^2$) and remained at this low value for several months. On one occasion, the corrosion current density dropped to a low value (0.1 $\mu A/cm^2$) and then increased about 100 $\mu A/cm^2$ (Fig. 3). This behavior was associated with a rupture in the iron sulfide film (Fig. 4).

If ferrous ions (ferrous ammonium sulfide) were added to the medium, the corrosion rate increased (Fig. 3) before decreasing as had previously been observed by Adams and Farrer (15).

These observations suggested that the ferrous ions were inhibiting film formation and permitting some corrosive metabolite to come in contact with the iron surface initiating corrosion. A culture of the marine sulfate-reducing organism, Seitz-filtered to remove any bacterial cells and rendered free of excess sulfide ions by addition of ferrous ions, was found by Iverson (21,24) to increase greatly the corrosion rate of mild steel after an induction period between three and four days (Fig. 5). The corrosion rate after 14 days was about 25 times that of the rate of steel corrosion in oxygenated sea water. Analysis of the corrosion products by the technique described previously again indicated iron phosphide as the main corrosion product. A view of the corroded iron surface is shown in Fig. 6.

It was suggested by Iverson (21) that these observations indicated the formation of a highly corrosive metabolic product containing phosphorous which upon access to the base surface of iron initiated a general type of corrosion. If, however, the iron surface was covered with a film of iron sulfide, the corrosion process would be inhibited. Thus, the fate of iron in contact with sulfate-reducing bacteria under anaerobic conditions would depend on which metabolite reached the iron surface first. If the corrosive metabolite reached the surface first corrosion would occur, but if hydrogen sulfide was the first metabolite at the surface and formed a film, corrosion would be hindered until the iron sulfide film broke down. Such a behavior according to Iverson (21) would explain the erratic results observed by others in the field and in the laboratory. Positive proof for this metabolite mechanism will await the identification of the corrosive metabolite.

ACKNOWLEDGMENT

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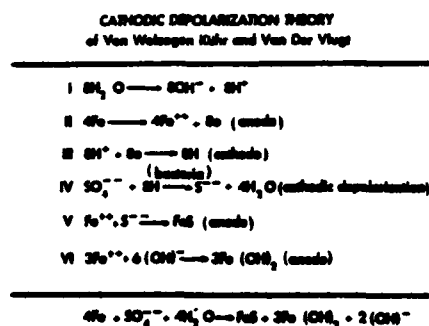   Fouling, Northwestern Union Press, Evanston, IL, p. 61 (1972).

Fig. 1. Equation I - The ionization of
water, Equation II - The ioni-
zation of iron (corrosion),
Equation III - The formation of
hydrogen, Equation IV - The re-
moval of hydrogen (electrons)
by the bacteria causing iron to
corrode (Equation II), Equations
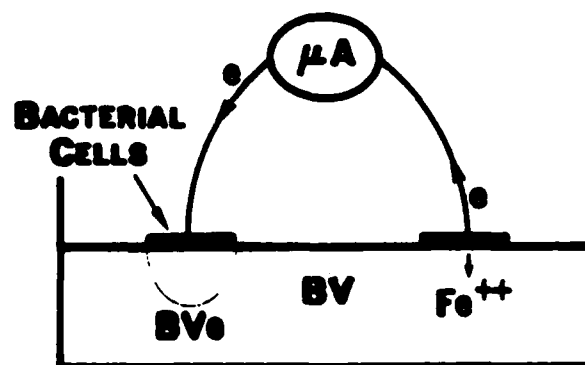V and VI - Secondary reactions.

Fig. 2. Measurement of "cathodic depolari-
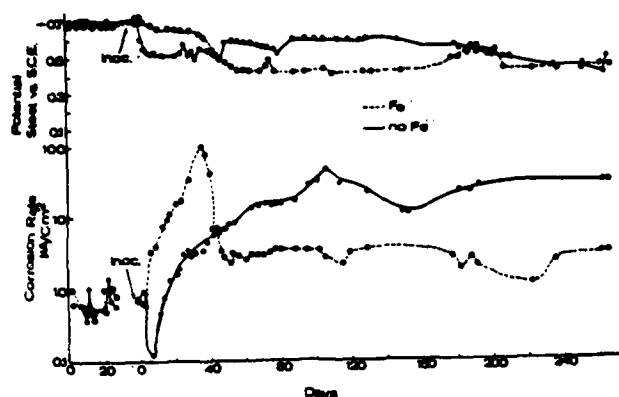zation current."

Fig. 3. Corrosion current density and potential (vs. standard calomel electrode) of mild steel (1020) in aged sea water containing Trypticase (1.5%), Phytone (0.5%) and sodium chloride (0.5%) with, and without the addition of Fe ion (0.25% ferrous ammonium sulfate). Inoculation after 26 days with culture of Desulfovibrio (marine strain). Inoculum consisted of 0.5 ml suspension of cells in sterile aged sea water from a six day old plate of Trypticase-Phytone--sea water medium.
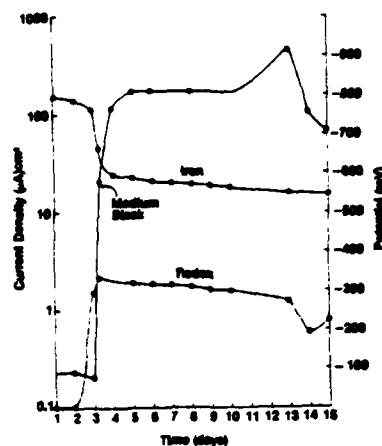

Fig. 5. Corrosion current density and potential (vs. S.C.E.) of mild steel (1020) electrode in culture filtrate (Seitz) of nine day old Trypticase-Phytone-sea water culture of a marine strain of Desulfovibrio. Sulfide ions were removed by the addition of excess ferrous ions.
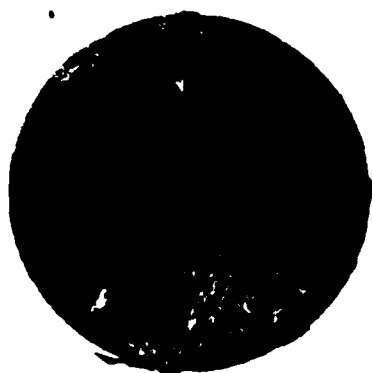

Fig. 4. Face of 1020 steel electrode (1/4" dia) after removal from Trypticase-Phytone sea water medium showing dark film and areas at top edge where the iron sulfide film has broken away from the surface. Area at bottom due to drop of KCl-agar which fell on specimen from Luggin capillary (10X mag.).

Fig. 6. Electrode surface of steel electrode (1/4" dia) after removal from the filtrate.

MATERIALS AND PROCESSING PROBLEMS ASSOCIATED WITH SULFATE-REDUCING BACTERIA IN PETROLEUM RECOVERY

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INTRODUCTION

Sulfate-reducing bacteria cause costly deterioration problems in many phases of petroleum recovery, largely as a result of the production of hydrogen sulfide. The corrosion of iron and steel in the form of storage tanks, pipelines, pumps, etc. is perhaps one of the greatest problems caused by these organisms because of the tremendous number of these items employed. Allred et al. (1) believed that sulfate-reducing bacteria were responsible for more than 77% of the corrosion occurring in one group of producing wells. Booth (2) attributed at least 50% of the failure of buried iron pipe in England to be due to bacterial action. Hydrogen sulfide attacks a wide range of equipment in the petroleum industry including steel casings, pumps, oil tanks, gas holding tanks, as well as refining equipment. The main types of corrosion attributed to hydrogen sulfide includes hydrogen embrittlement, blistering, and stress corrosion cracking.

Sulfate-reducing bacteria also create plugging problems in underground petroleum reservoirs, hampering the secondary recovery of oil by water injection (3). The bacterially produced hydrogen sulfide reacts with dissolved iron to form iron sulfide precipitates,·resulting in plugging of the injection well and reduction of oil production. These bacteria may also form calcium carbonate precipites. Sulfate-reducing bacteria have recently been implicated in the failure of tertiary oil recovery operations, possibly by degrading polymers used in situ as mobility control agents (4). Thus, many aspects of petroleum recovery are hindered by the activity of these organisms.

Sulfate-reducing bacteria are strict anaerobes that perform anaerobic respiration, oxidizing certain organic compounds or hydrogen and reducing sulfate (and sometimes other reduced sulfur compounds) to hydrogen sulfide. They are commonly found world-wide in groundwaters and are often found associated with petroleum deposits. These bacteria have been detected in waters from great depths, up to several thousand meters below the earth's surface, at temperatures approaching the boiling point of water (5).

Historically, sulfate-reducing bacteria were thought to grow at the expense of only a few simple organic compounds and hydrogen. Recent work, however, has shown that sulfate-reducing bacteria can oxidize a wide variety of organic compounds including alcohols, organic acids, long chain (up to C18) fatty acids, and aromatic compounds. Several new species and genera of sulfate-reducing bacteria have been described as a result of these findings. Much of this important work was done by Widdel (6) and it has made a significant impact in our evaluation of the role sulfate-reducing bacteria in the anaerobic degradation of

organic material. Obviously, these findings will affect research directed toward the control of these organisms in the petroleum industry.

Throughout the years a number of inhibitors have been tested (see ref. 7) in attempts to control the growth of sulfate-reducing bacteria. Aeration and acidification treatments have also been undertaken (8). Generally there has been only sporadic success in controlling these organisms in the field. A more promising approach to the problem has been presented by Ruseska et al. (9) who tested biocides under field conditions using a pipe with removable studs that could be examined for colonizing bacteria. In natual waters, microorganisms are generally found attached to surfaces. Penetration of biocides through a biofilm on a pipe surface may be greatly limited, consequently laboratory data on biocide testing may not reflect real-world efficacy. Biocides capable of penetrating biofilms may prove to be the most successful agents for control of sulfate-reducing bacteria.

MICROBIAL ANAEROBIC CORROSION MECHANISMS

In addition to the corrosive effect of hydrogen sulfide occurring under acidic conditions, both in the presence and absence of air, a considerable amount of corrosion occurs under anaerobic conditions in the neutral pH range. This anaerobic corrosion of iron has been the subject of many investigations for decades. Nearly 50 years ago von Wolzogon Kuhr and van der Vlugt proposed the cathodic depolarization theory to explain the anaerobic corrosion of iron (10). In this theory, sulfate-reducing bacteria were considered to play a prime role in corrosion by removing hydrogen (and reducing sulfate to sulfide) from the metal surface at a cathodic area, thus depolarizing the iron and causing ferrous ions to go into solution. Secondary reactions occur between the ferrous ions and sulfide and hydroxide ions. The overall reaction is:

$$4Fe + SO_4 + 4H_2O \rightarrow FeS + 3Fe(OH)_2 + 2(OH)^-$$

However, the rate of corrosion of iron by cathodic depolarization is too small to account for the high corrosion rates observed in natural environments (11). Other mechanisms are required to explain anaerobic microbial corrosion.

Iverson (11) has provided evidence for the production of a highly corrosive volatile metabolite containing phosphorus, and possibly sulfur, produced by sulfate-reducing bacteria. Current studies in our laboratory are therefore directed toward characterization of corrosive metabolites produced by sulfate-reducing bacteria.

LABORATORY STUDIES

In this preliminary communication we report on the production of volatile organosulfur compounds by sulfate-reducing bacteria. Two strains of sulfate-reducing bacteria were used in the study, 1) the American Petroleum Institute mid-continent A strain of Desulfovibrio desulfuricans (designated the API strain) and an organism isolated from corroded steel pilings on the coast of Virginia tentatively identified

as <u>D</u>. <u>desulfuricans</u> (designated the marine strain). The organisms were
maintained at 28 °C on trypticase soy (TSY) agar (made with aged
seawater) under a hydrogen atmosphere. Headspace gas above cultures
growing on TSY agar slants or plates was analyzed by flame photometric
gas chromatography (GC-FPD) (12) for selective detection of volatile P
and S compounds. The volatile S compounds were separated on a 6 ft x
1/4 in teflon column packed with chromosil 330 (Supelco). A temperature
program of 25 °C for 4 min followed by heating at 32 °C/min to 60 °C
was used. High purity nitrogen at a flow rate of 20 mL/min was used
as the carrier gas.

Both the API strain and marine strain produced primarily H_2S when
grown on TSY agar made with aged seawater (Fig 1). The API strain also
produced substantial CH_3SH, and the marine strain a small amount of
CH_3SH on this medium. On TSY agar made with deionized water, the API
strain produced very little H_2S, the primary volatile S product being
CH_3SH. A trace of $(CH_3)_2S_2$ was also detected. The amount of $(CH_3)_2S_2$
increased with incubation time, perhaps as a result of CH_3SH oxidation
(13). The marine strain did not grow on TSY made with deionized water.
The deionized water medium contained very little sulfate compared
to the seawater medium, and, since sulfate serves as the respiratory
electron acceptor in sulfate-reducing bacteria (8), it is not surprising
that a variation in the amount of H_2S produced by the API strain was
observed. Good growth of the API strain occurred on both media,
however. The physiological mechanism of CH_3SH production in these .
organisms has not yet been determined. However, many microorganisms
are known which produce CH_3SH from the decomposition of methionine (13).
We screened CH_3SH for corrosive action on steel coupons. One mL
of authentic CH_3SH was added to a capped test tube containing a coupon
of 1010 mild steel in 8 mL of an anoxic solution of 1% yeast extract
under a hydrogen atmosphere. Only after several weeks of incubation
did we observe very slight corrosion, indicating that CH_3SH alone was
not responsible for the rapid corrosion of steel observed previously
with spent culture medium (11).
Our investigation into the production of corrosive metabolites by
sulfate-reducing bacteria and of the physiological basis for production
of volatile organosulfur compounds by these bacteria is continuing.

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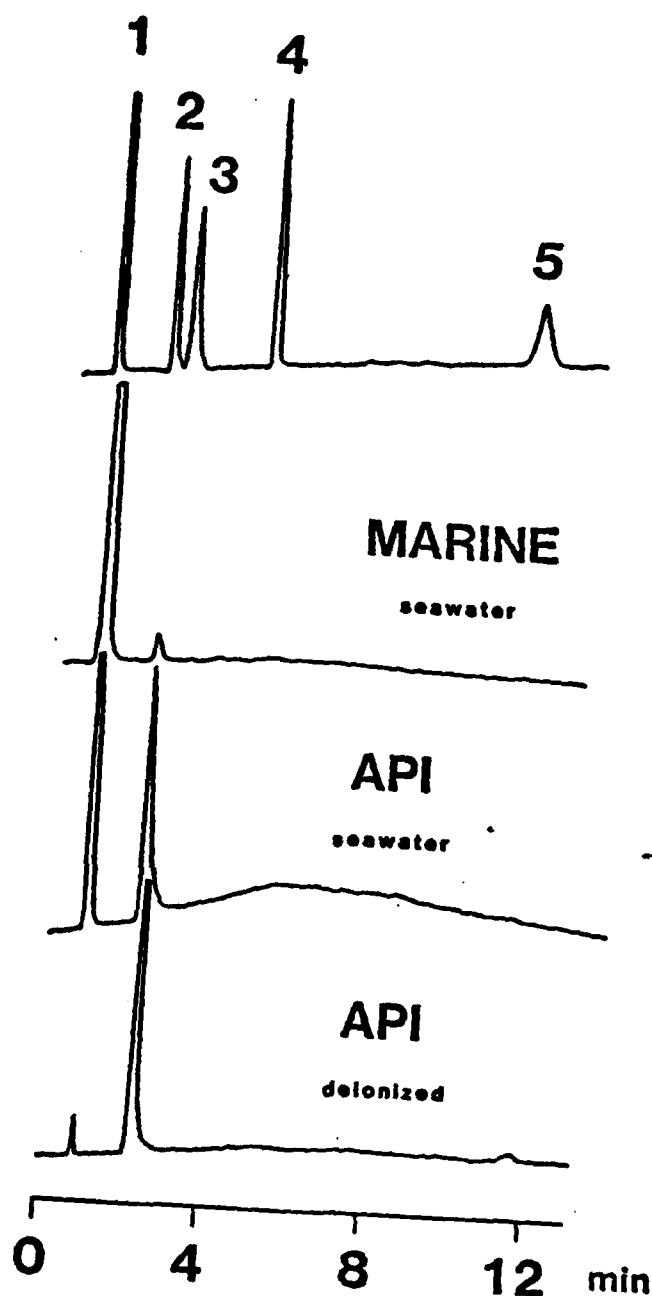13. Segal, W., and R.L. Starkey, J. Bacteriol. 98, 908 (1969).

Figure 1. Production of volatile sulfur compounds by the API and marine strains of D. desulfuricans. Headspace gas above 96 hour old cultures growing on TSY agar (made with deionized water or aged seawater) was analyzed by GC-FPD. Standards: 1, H_2S; 2, CH_3SH; 3, CS_2; 4, $(CH_3)_2S$; 5, $(CH_3)_2S_2$.

ANAEROBIC CORROSION BY SULFATE-REDUCING BACTERIA
DUE TO A HIGHLY-REACTIVE PHOSPHORUS COMPOUND

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SYNOPSIS

Significant corrosion products, formed in the
bacterially induced, anaerobic corrosion of
iron, appear to be iron phosphides in addition
to iron sulfide. Upon acidification, these
products release PH_3 and H_2S respectively, which
can be detected by gas chromatography employing
a flame photometric detector (GC-FPD). The iron
phosphide can be formed by the action of a
volatile phosphorus-containing compound produced
during the growth of cultures of Desulfovibrio.
In addition to the production of this phosphorus
compound and hydrogen sulfide, the organism also
produces two relatively non-corrosive (to iron)
compounds: methylmercaptan and dimethyldi-
sulfide. The bacterial anaerobic corrosion of
iron was partly simulated by a phosphorus com-
pound produced during the reaction of H_2S with
hypophosphite in aqueous solution. Iron phos-
phide was formed as a corrosion product. A
volatile phosphorus compound was produced by the
action of hydrogen sulfide on crystals of hypo-
phosphite, phosphite and phosphate. Although
this volatile compound could not be detected
with the GC-FPD (phosphorus mode) its presence
was indicated by absorbing the compound in
acidified permanganate solution and detecting
the presence of phosphorus by the phospho-
molybdate reaction. Traces of phosphine were
also detected, as a possible degradation com-
pound in the headspace above the three phos-
phorus compounds after a few weeks.

INTRODUCTION

The study of anaerobic corrosion was initiated in 1934 when von Wolzogen Kühr and van der Vlugt provided significant evidence that anaerobic corrosion was caused by the activities of sulfate-reducing bacteria.[1] They also proposed a theory to account for this phenomenon, namely the cathodic depolarization theory. This theory was partly based on the findings of Stephenson and Stickland[2] that the sulfate-reducing bacteria utilized molecular hydrogen in their metabolism. According to the theory, the organisms remove hydrogen (electrons) from the surface at cathodic areas causing iron to corrode, that is, go into solution as ferrous ions at anodic areas.

Evidence for and against this theory forms much of the literature on microbial corrosion. A considerable number of studies of this type of corrosion were carried out by electrochemical techniques at the National Chemical Laboratory at Teddington, England (presently the National Physical Laboratory) by Booth and Tiller and their associates and by Miller and co-workers at Manchester University.[3] One fact which emerged from these studies was that the stimulation of anaerobic corrosion rates was caused by the presence of ferrous ions.

A number of modifications to the cathodic depolarization theory have been proposed in the last 15 years. Mara and Williams[4] proposed that bulk iron sulfide was involved in cathodic depolarization, acting as a cathode and absorbing hydrogen in proportion to the cationic defects in the iron surface. King and Miller[5] postulated that the bacteria on the surface of the ferrous sulfide continually regenerated or depolarized the iron sulfide by removal of the hydrogen as a result of their hydrogenase activity. Miller[3] suggested that the newly formed iron sulfide, as a result of its physical movement by the bacterial cells, caused fresh iron sulfide surfaces to be constantly brought into contact with steel. Costello[6] proposed that the cathodic depolarizing activity of the sulfate-reducing bacteria was due to the cathodic activity of the hydrogen sulfide.

In an effort to model the cathodic depolarization theory, Iverson[7] used a system involving two mild steel electrodes placed on the surface of agar plates. He employed benzyl viologen (BV) as an electron acceptor, in place of sulfate, in an organically buffered agar (pH 7 ± 0.2). One electrode was placed on the surface of the agar with a mass of hydrogenase-positive cells of <u>Desulfovibrio desulfuricans</u> (Mid Continent A strain, referred to as the American Petroleum Institute or API strain) underneath. The electrode was electrically connected to a second electrode resting on the agar surface (no bacterial cells between electrode and the agar). After remaining for several hours (<u>ca</u>. 17 hours) in an inert atmosphere (helium or argon), it was found that the BV underneath the electrode, in contact with the cells, was reduced (violet color). Ferrous ions had gone into solution in the agar underneath the second electrode. A sustained current density, up to 9 hours (<u>ca</u>. 1 $\mu A/cm^2$) could be measured between the electrode.

Thus a working model of corrosion for the cathodic depolarization theory (fig. 1) was obtained, but not completely. The current density could not explain the high rates of corrosion observed in the field and secondly, if sulfate was employed as an electron acceptor instead of BV, no sustained "cathodic depolarization" current could be obtained, nor did the iron go into solution in the agar at the anode as was the case with BV. These results tentatively suggested that the cathodic depolarization theory could not explain the severe anaerobic corrosion in the field and that another mechanism must be involved since sulfate did not appear to act as an acceptor for bacterial cells in contact with iron.

Evidence is presented that the primary cause of anaerobic corrosion is due to a volatile, highly corrosive product containing phosphorus, which is produced from the action of bacterially produced hydrogen sulfide on inorganic phosphorus compounds in the environment in which sulfate-reducing organisms are growing or metabolizing. Direct bacterial formation of this compound may also occur.

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EVIDENCE FOR A NOVEL MECHANISM

The following evidence is presented to support the above concept of microbially produced anaerobic corrosion by a volatile phosphorus compound.

A. The Corrosion Product is Iron Phosphide

If, in the model described above for demonstration of cathodic depolarization, yeast extract, with and without added sulfate, was incorporated in the agar (providing conditions for cell growth) to replace the buffer and BV, no effect similar to the results obtained with BV was again noted. A blackened area in the agar (with and without added sulfate) was observed only under the electrode in contact with the bacterial cells (Iverson,[8]). Ferrous ions were noted in this area upon development with ferricyanide.

To repeat the development of this blackening in liquid medium, a sterile coupon or mild steel was placed in a sterile one percent yeast extract solution, with the pH adjusted to 7.0 ± 0.2, and a heavy suspension of Desulfovibrio API strain cells was added. Under a hydrogen atmosphere, blackening of the yeast extract solution occurred within a few hours. Upon removal of this black precipitate by centrifugation and introduction of a second steel coupon in the supernatant, blackening of the solution was again observed. This was repeated a third time. The three black centrifugates were collected and dried under vacuum. X-ray powder pattern analysis of the product (originally amorphous), heated in a vacuum oven to 1230 °C and allowed to cool for 24 hours, indicated the presence of magnetic iron phosphide (Fe_2P).[8]

This result raised the question of whether iron phosphide is a corrosion product which occurs under conditions in the field.

Since amorphous iron phosphide when acidified, yields phosphine (PH_3) as a gaseous product, we have examined a few acidified black anaerobic corrosion products from various sources, including material from tubercles inside water pipes, for the presence of PH_3. Small amounts of the black corrosion products were placed in Hungate tubes (screw capped tubes

with rubber septa in the screw caps), the air replaced with hydrogen, and a small amount of deaerated sulfuric acid (10 percent V/V H_2SO_4) added. The headspace over the dissolved corrosion product was then examined using a gas chromatograph with a flame photometric detector (FPD) (phosphorus mode). A comparison standard of phosphine was prepared by acidifying zinc phosphide and maintaining the gas in a vial equipped with a Teflon Mininert valve. Figure 2 (top) shows a chromatogram of PH_3 and H_2S. Time course studies have shown that iron phosphide dissolves more slowly than iron sulfide, the height of the PH_3 peak first appearing smaller than the H_2S peak, which reaches a maximum peak height within a few minutes after acidification. Examination of a significant number of corroded field samples will be necessary to establish the presence of iron phosphide as a universal anaerodic corrosion product.

Gaylarde[9] has also mentioned that phosphorus occurs in quite high levels in black corrosion deposits covering anaerobically corroded steel as indicated by Electron Spectroscopy for Chemical Analysis (ESCA) studies.

In the field, anaerobic corrosion is usually detected by adding a few drops of acid (usually diluted HCl) to the black corrosion products and testing the resulting gas for the presence of hydrogen sulfide, using lead acetate paper. Usually the odor of hydrogen sulfide can also be detected. It was noted by Romanoff[10] at the National Bureau of Standards that, occasionally, blackening of lead acetate paper occurred without any detectable odor of hydrogen sulfide. It should be noted that phosphine also produces blackening of lead acetate paper, but primarily around the edges. These observations are further suggestive evidence for the presence of iron phosphide as a corrosion product.

In the original report by von Wolzogen Kühr and van der Vlugt,[1] no mention was made of iron phosphide in their analysis of corrosion products. It is the authors' belief that iron phosphide was present but due to their technique, they failed to detect it. The technique, which they used, involved acidifying the black, anaerobic corrosion products and passing the liberated gas through a solution of silver nitrate. In addition to forming silver sulfide,

silver phosphide could also have been formed. Phosphine will react to silver nitrate to form black silver phosphide:

$$3 AgNO_3 + PH_3 \rightarrow Ag_3P + 3 HNO_3.$$

As they reported that "measurement of the sulfide in the fluid took only a couple of hours, while for the pieces of iron it lasted some six hours" it is quite suggestive that they were dissolving the more resistant iron phosphide rather than the less resistant iron sulfide.

B. <u>Other Phosphorus-Containing Products Produced as a Result of the Activities of Sulfate-Reducing Bacteria</u>

The formation of iron phosphide suggested that a phosphorus compound was involved in its formation and that other phosphorus compounds might also be formed as a result of its activities. Hallberg,[12] for example, reported that samples from a continuous culture of sulfate-reducing bacteria at 25 °C and PH $\approx$ 8, as well as subsamples heated in evacuated silica tubes at 120 °C, yielded a new phosphate compound estimated to be:

$$KNa_3(Fe_{1.5} - Mg_{2.5})(PO_4)_3(OH)_3$$
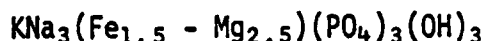
Suess[13] in investigating the mineral phases formed in recent anoxic sediments of the Baltic Sea, indicated the presence of two unknown phosphate compounds by a fractional leaching of the sediment with increasing H^+ ion concentration.

Further, upon exposure of mild steel coupons for one month, in a one percent yeast extract solution containing large numbers of <u>Desulfovibrio</u> cells, where iron phosphide formation had occurred, Iverson[14] observed the formation of vivianite crystals $[Fe_3(PO_4)_2 \cdot 8 H_2O]$ on the coupons. In relation to this observation, Roman nails unearthed from a bog in England, where sulfate-reducing activity was present, were found to be coated with vivianite (Booth <u>et al.</u>,[15]). This coating was believed to have afforded protection to the nails.

C. Rapid Corrosion in the Absence of Both
   Bacterial Cells and Hydrogen Sulfide
   by a Phosphorus Compound Produced in
   Cultures of Desulfovibrio

Corrosion rates of mild steel (1020) in an
aged sea water medium containing enzymatic
digests of casein and soybean meal by a marine
strain of Desulfovibrio, isolated from steel
piling off the Virginia Coast, were reported by
Iverson[16] using polarization techniques. He
found that the corrosion current density gen-
erally decreased to a very low value (0.1 to
0.5 $\mu A/cm^2$) and remained so for several months.
On one occasion, the corrosion current density
dropped to a low value (0.1 $\mu A/cm^2$) and then
increased to about 100 $\mu A/cm^2$ (fig. 3). This
behavior appeared to be associated with a
rupture in the iron sulfide film.

As the presence of ferrous ions in stim-
ulating corrosion has previously been mentioned,
their presence in the sea water medium also was
found to cause the corrosion rate to increase
(fig. 3). These observations suggested that the
ferrous ions were inhibiting film formation and
permitting a corrosive material to come in
contact with the iron surface. A culture (nine
days old) of the organism was rendered free of
excess sulfide ions by the addition of excess
ferrous ions and Seitz-filtered to remove the
iron sulfide precipitate as well as the bac-
terial cells (Iverson[16]). It was found that the
corrosion rate after an induction period between
three and four days, increased very rapidly to
about three logs of current density (fig. 4) in
a few days. The corrosion rate at 14 days was
about 25 times that of the corrosion rate of
steel in oxygenated sea water. Such corrosion
rates could account for the very rapid anaerobic
corrosion noted in the field. Analysis of the
corrosion products by the previously described
technique of vacuum oven heating again indicated
that iron phosphide was a corrosion product
(Fe_3P in this case) in addition to FeS (troilite).

D. Production of a Volatile, Corrosive,
   Phosphorus-Containing Compound by
   Desulfovibrio

It was noted several years ago[17] that a
volatile phosphorus-containing compound was

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produced by both the marine strain and the API strain of <u>Desulfovibrio</u>. The headspace above agar plate cultures of the organisms was passed through an acidified permanganate trap, which oxidizes hydrogen sulfide to sulfate and phosphine to phosphate, according to the procedure of Borrowdale and Shanahan.[18] The presence of phosphate was indicated upon completion of the phosphomolybdate reaction; the water blank indicating only a trace of phosphate.

Initially, the gas was suspected of being phosphine which was demonstrated to be non-corrosive to iron. Phosphine may be identified by its reaction with mercuric chloride to form a yellow precipitate $[P(HgCl)_3]$ identifiable by the X-ray diffraction technique according to Moser and Brukl,[19] Lemhoult,[20] Wilmet,[21] and Beyer.[22] This was confirmed at NBS on a sample prepared by reacting phosphine with mercuric chloride.

The compound which formed in a one percent $HgCl_2$ solution placed above agar plates (two percent yeast extract plus dipotassium phosphate; 0.25 g/100 ml agar) inoculated with the API strain, however, formed a tan-colored precipitate. Analysis of this compound by X-ray diffraction indicated the presence of another compound with certain similarities to Hg_3PO_4.

Six small iron nails (12 mm in length) in sterile aged sea water medium containing Trypticase and Phytone (three nails/dish) and ferrous ammonium sulfate (to remove any H_2S) were placed over nine inoculated agar plate cultures of the marine strain. The agar medium in the Petri plates contained the same materials as the sea water medium, including ferrous ammonium sulfate. After a month incubation at 26 °C, the total weight loss of the six nails was 20.8 mg for a 2.9 percent total weight loss (total weight of six nails-700 mg). One nail had lost about 6.6 percent of its total weight. A black precipitate formed around the nails in the bottom of the dishes. Acidification of the corroded nails indicated the presence of PH_3 in addition to H_2S (fig. 2 bottom two chromatograms).

Examination of the headspace above agar surface (slant and plate) cultures (API and marine strain) as well as liquid cultures (Trypticase Soy Agar and Broth; aged sea water

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and deionized water) using the GC-FPD (phosphorus mode) for a novel phosphorus compound did not reveal any such compound. Occasionally, however, traces of phosphine were detected in the headspace above agar slant cultures of both strains.

With the detector in the sulfur mode, two sulfur compounds (methyl mercaptan and dimethyldisulfide) were tentatively identified by their retention times in comparison with the retention times of authentic reference compounds (fig. 5). Carbon disulfide was also identified in the headspace of the cultures, but this is believed to have come from the rubber septum of the cap of the Hungate tubes since uninoculated controls and capped empty Hungate tubes also indicated the presence of CS_2. Methyl mercaptan and dimethyldisulfide were found to be relatively non-corrosive, individually and combined to coupons of mild steel in a one percent yeast extract solution atmosphere. Some blackening of the solution was noted and below the surface of the medium after a few weeks at room temperature, however.

E. Simulation of Anaerobic Corrosion with Iron Phosphide Formation by a Mixture of Hydrogen Sulfide and Hypophosphite

As a volatile phosphorus compound appeared to be produced during the growth of Desulfovibrio which formed iron phosphide, it was thought that this volatile compound might be a reduced form of hypophosphite (PO_2^{3-}).

After several attempts to reduce hypophosphite by metals and redox dyes, hydrogen sulfide was suggested as a possible reductant by the following equation from a paper by Kuever and Stahl:[23]

$$H_3PO_2 + 2H_2S \rightarrow PH_3 + 2 H_2O + 2S.$$

No evidence, however, to support this reaction nor any reference to this reaction was mentioned in the paper.

The blackening of a solution (8 ml) of one percent hypophosphite $(NaH_2PO_2 \cdot H_2O)$ in deionized water containing a mild (1010) steel coupon (51 x 9 x 0.5 mm) was used as an indication of corrosion intensity. The addition of hydrogen

sulfide (1 cc) caused considerably more blackening of the solution than did either of the control tubes (deionized water and H_2S; deionized water plus one percent hypophosphite). The black precipitate upon acidification produced only H_2S, however. (The precipitate was filtered through a 0.45 μm millipore filter, placed in a Hungate tube, the atmosphere replaced with hydrogen and 1 ml deaerated 10 percent (v/v) H_2SO_4 added).

Extensive corrosion similar to the complete blackening of the tubes, observed in the case where the bacteria were involved, evidently was prevented by the film of hydrogen sulfide which probably formed immediately before the suspected phosphorus compound could be formed.

To allow formation of the suspected phosphorus compound to occur, H_2S (1 cc) was added to 8 ml of a one percent hypophosphite solution without any coupon being added. A slight cloudiness of the solution was noted after an hour, due to formation of a white precipitate. After 24 hours a calculated amount of ferrous ions (deaerated ferrous ammonium sulfate) was added to the solution to remove the sulfide ions. No black precipitate was noted, however, as is the case when the solution contains organic material (viz. yeast extract). After a second period of 24 hours, small nails (12 mm) were added to the tubes and the atmosphere replaced with hydrogen. The reaction was allowed to proceed for almost two months. During this time, trains of small bubbles, possibly hydrogen, were observed to come from the nails from one or two areas. No phosphine could be detected in the headspace. A black precipitate developed in the tubes during this interval. This precipitate from two tubes was filtered and acid treated. Again H_2S was present, but no PH_3. The nails (0.121 g and 0.149 g) which had a dark coating, and had lost 1 and 1.2 mg weight respectively, were acidified under a H_2 atmosphere. Relatively large amounts of PH_3 and H_2S were detected in the headspace by the GC-FPD. The PH_3 gradually increased over the 24 hour period while the H_2S peak initially reached a maximum in a few minutes, and then slowly decreased during this time. Control nails, directly from the package, after acetone degreasing shows no evidence of either PH_3 or H_2S upon acidification. This was a preliminary

experiment and no control nails were exposed separately to either H_2S or hypophosphite in deionized water. No blackening of steel coupons in hypophosphite solution has ever been observed, however. The results are quite suggestive in that a layer of iron phosphide was produced on the surface of the nails as a result of a phosphorus intermediate formed from the action of H_2S on hypophosphite. In addition, before concluding definitely that the severe anaerobic corrosion is caused by a phosphorus compound it will be necessary to use this system in organic media to duplicate the rapid and extensive corrosion obtained by the bacterial filtrate (minus sulfide ions) of the marine strain of Desulfovibrio, as previously mentioned.

F. Production of Volatile Phosphorus Compound by Action of H_2S on Phosphates, Phosphite, and Hypophosphite

In studying the reaction of hydrogen sulfide with inorganic phosphorus compounds in a non-aqueous system, evidence was obtained of the production of a volatile phosphorus compound. Hydrogen sulfide (1 cc) was added separately to crystals (15-20 mg) of hypophosphite ($NaH_2PO_2 \cdot H_2O$), phosphite ($Na_2HPO_3 \cdot 5H_2O$) and phosphate ($Na_3PO_4 \cdot 12H_2O$) in a hydrogen atmosphere. After 24 hours, 1 cc of headspace above the crystals was introduced into tubes containing deareated acidified permanganate solution with the presence of phosphorus indicated by a positive phosphomolybdate reaction. The crystals of phosphate and phosphite, but not hypophosphite, were observed to have become yellow in 24 hours. The yellow color disappeared upon exposure to air indicating that elemental sulfur was not formed. No volatile phosphorus compounds were observed by the gas chromatographic procedure with the flame photometric detector in the phosphorus mode. After one to two weeks, however, traces of phosphine were detected in the headspace above all three compounds.

In an aqueous system, the addition of hydrogen sulfide to concentrated or diluted solutions of hypophosphite resulted in the formation of water insoluble crystals which at the time of writing have not been identified.

Mass spectrometric studies underway (presumably the active compound is trapped in the chromatographic column) indicated that a phosphorus compound is formed over the crystals of hypophosphite in the presence of H_2S. As the

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compound is formed in relatively small amounts compared to the hydrogen sulfide, unequivocal results have not been presently obtained. In subsequent experiments it is planned to remove the hydrogen sulfide from an aqueous system and collect the phosphorus compound in a cold trap.

SUMMARY

Based on the results presented, some of which are preliminary, the following conclusions are presented:

1. Iron phosphide appears to be a corrosion product in the microbial, anaerobic corrosion of iron both in laboratory and field samples.

2. A phosphorus-containing product is assumed to be produced, as a result of the activities of sulfate-reducing bacteria, which reacts with iron to produce iron phosphide.

3. From evidence of novel and well-known phosphorus compounds which are formed in the immediate vicinity of sulfate-reducing bacteria, the phosphorus-containing product appears to be involved in chemical reactions where these phosphorus compounds are formed.

4. The phosphorus compound produced by sulfate-reducing bacteria involved in iron corrosion is volatile. No direct contact between the bacteria or bacteria-free culture filtrates is required for anaerobic corrosion.

5. A volatile phosphorus compound is also produced by the action of hydrogen sulfide on phosphate, phosphite, and hypophosphite.

6. The volatile phosphorus compound appears to be too reactive to survive passage through the chromatographic column which was employed.

7. Iron phosphide was produced by the action of hydrogen sulfide on hypophosphite in the presence of iron, as evidenced by the liberation of phosphine from acidified iron specimens.

8. The volatile phosphorus compound appears to decay slowly, liberating phosphine which can be detected using flame photometric gas chromatography.

From the evidence discussed, it appears that anaerobic corrosion by sulfate-reducing bacteria is caused by a highly active volatile phosphorus compound which reacts with bulk iron to form iron phosphide. This preliminary evidence also indicates that the volatile phosphorus compound may also be produced by the direct action of bacterially produced hydrogen sulfide on inorganic phosphorus compounds. According to this concept, any organism that produces hydrogen sulfide, under anaerobic conditions, in the presence of certain phosphorus compounds should stimulate the corrosion of iron, providing the iron does not have a film of iron sulfide present.

A summary of this concept and the findings reported are presented in schematic form in fig. 6. If, under anaerobic conditions, bacterially produced hydrogen sulfide first comes in contact with an iron surface, a layer of iron sulfide will be formed which inhibits further corrosion. If sulfide ions, however, are trapped, for example, by an excess of ferrous ions in the vicinity of the iron so that film formation is prevented, or if the unstable iron sulfide film breaks down, a volatile phosphorus compound, produced by the action of hydrogen sulfide on phosphorus compounds, will corrode the iron, yielding iron phosphide as a corrosion product. For unequivocal proof of this mechanism, it remains to be demonstrated that severe anaerobic corrosion of iron can be produced by the phosphorus compound in the absence of hydrogen sulfide. It also remains to be determined whether this volatile phosphorus compound may also be produced intracellularly by the bacterial cells.

In addition to the metabolic production of hydrogen sulfide by sulfate-reducing organisms, methylmercaptan and/or dimethyldisulfide are/is produced.

Certain commercial equipment, instruments, or materials are identified in this paper to adequately specify the experimental procedure. Such identification does not imply recommendation or endorsement by the National Bureau of Standards, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

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APPENDIX

Chromatographic procedure--The chromatograph
employed a Chromosil 330 (Supelco, Inc.,
Bellefonte, Pennsylvania) column (Teflon). The
N_2 carrier flow was 60 ml/min. Oven
temp.: 25 °C; injection port temp., 150 °C;
detector temp.: 200 °C. Flame photometric
detector. Phosphorus mode: filter, 526 nm; H_2,
200 ml/min.; air, 50 ml/min.; O_2, 20 ml/min.
Sulfur mode: filter, 394 nm; H_2, 50 ml/min.;
air, 50 ml/min. O_2, 20 ml/min.

CAPTIONS

1. Measurement of "Cathodic depolarization Current". BV-reduced benzylviologen--(violet). BV-oxidized benzylviologen--(colorless).
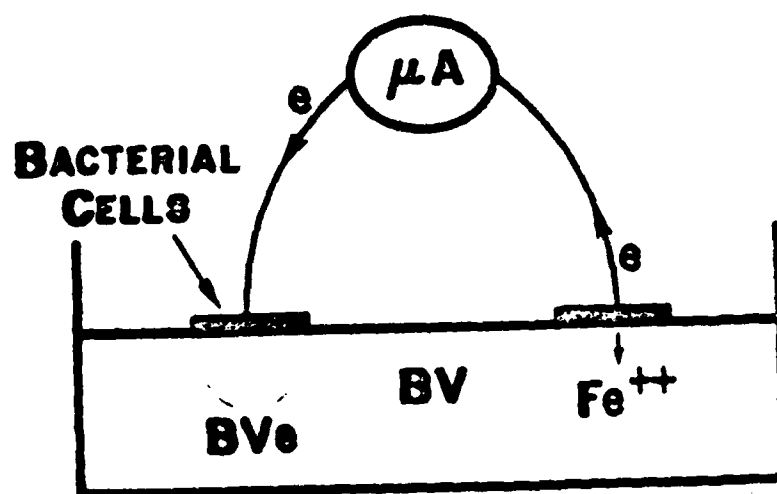
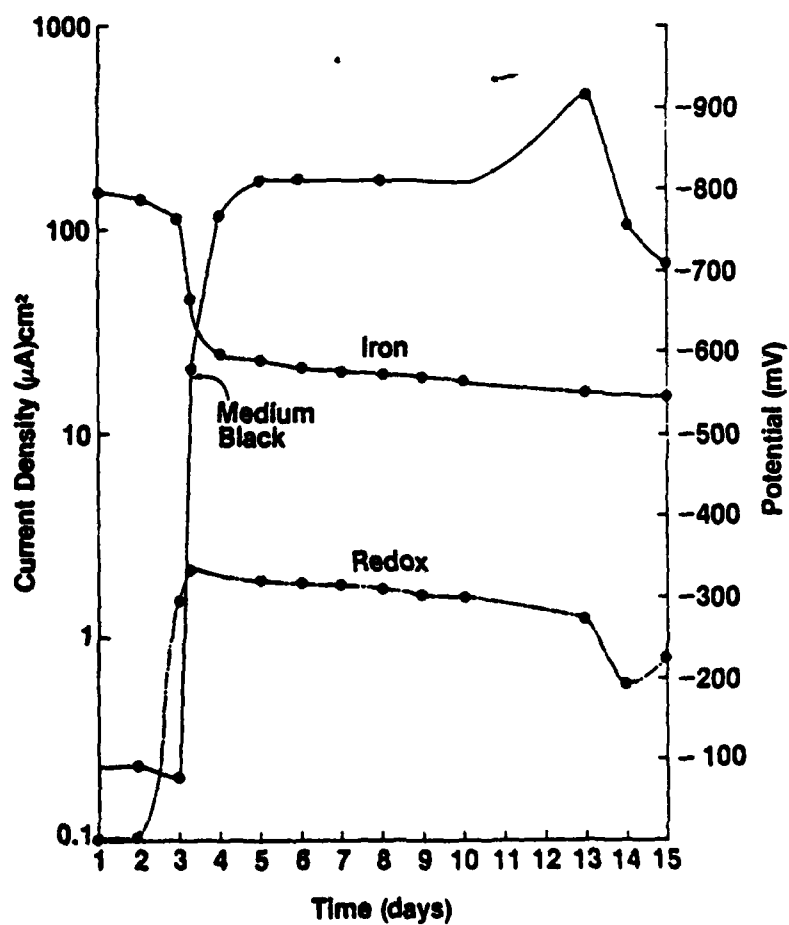2. Chromatogram of reference PH_3 and H_2S (phosphorus and sulfur modes) and acidified corroded nails exposed to headspace over culture of marine strain.
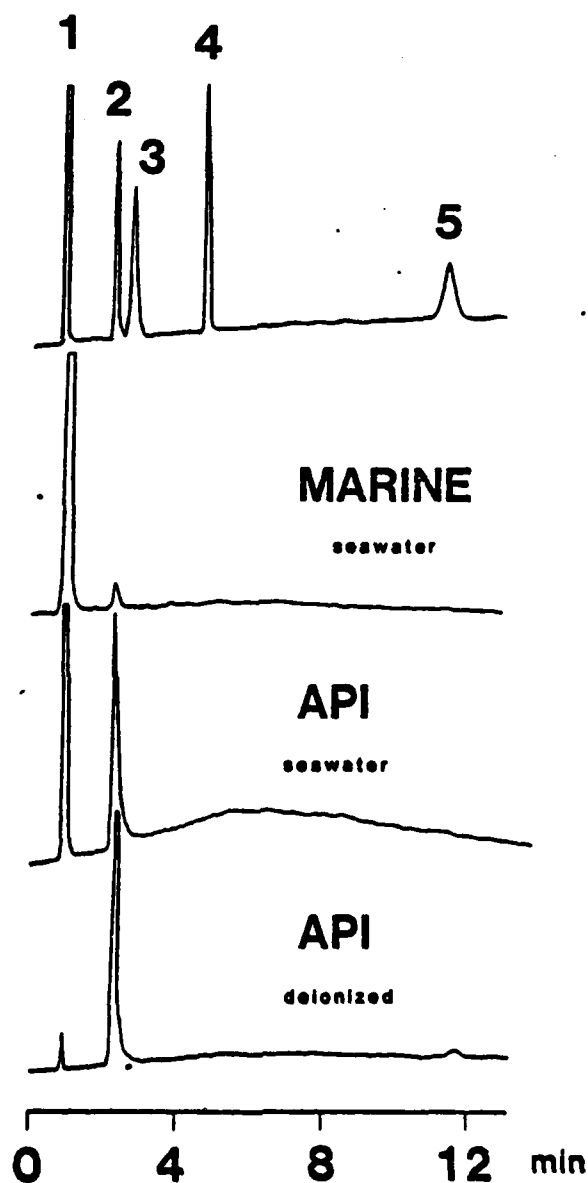
3. Corrosion current density and potential (vs. S.C.E.) of mild steel (1020) in inoculated agar sea water medium with and without Fe^{++} ions.

4. Corrosion current density and potential (vs. S.C.E.) of mild steel (1020) in culture filtrate (marine strain) minus cells and sulfide ions.

5. Chromatogram of volatile sulfur com pounds in headspace gas above growing API and marine strains. (Sulfur mode). Standards: 1, H_2S; 2, CH_3SH; 3, CS_2; 4, $(CH_3)_2S$; 5, $(CH_3)_2S_2$.

6. Schematic of anaerobic corrosion mechanism.

1. Measurement of "Cathodic depolarization
    Current". BV-reduced benzylviologen--
    (violet). BV-oxidized benzylviologen--
    (colorless).

Chromatogram of reference PH$_3$ and H$_2$S
(phosphorus and sulfur modes) and acidified
corroded nails exposed to headspace over
culture of marine strain.

3. Corrosion current density and potential (vs. S.C.E.) of mild steel (1020) in inoculated agar sea water medium with and without Fe^{++} ions.

19
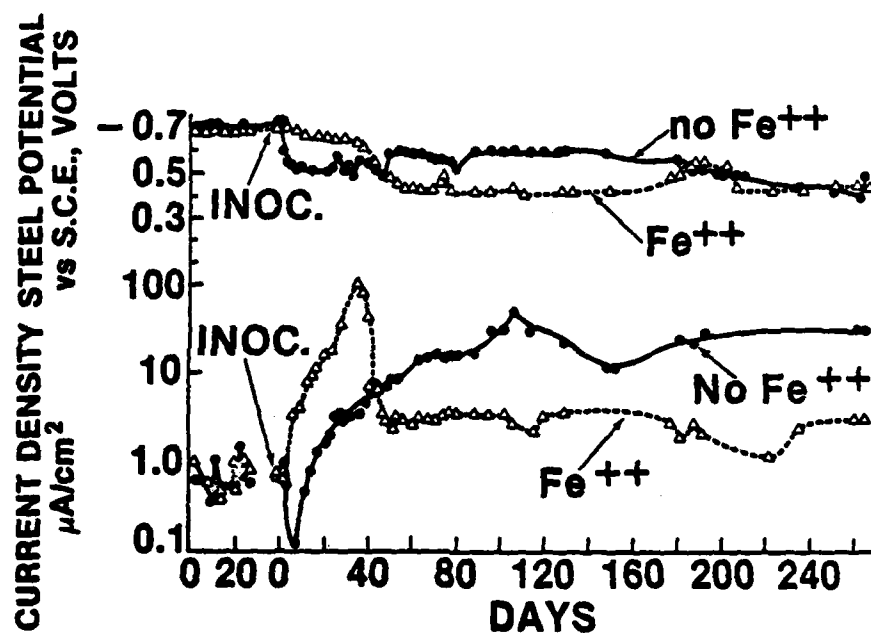
4. Corrosion current density and potential (vs. S.C.E.) of mild steel (1020) in culture filtrate (marine strain) minus cells and sulfide ions.

5. Chromatogram of volatile sulfur com
   pounds in headspace gas above growing
   API and marine strains. (Sulfur mode).
   Standards: 1, H_2S; 2, CH_3SH; 3, CS_2;
   4, $(CH_3)_2S$; 5, $(CH_3)_2S_2$.

6. Schematic of anaerobic corrosion mechanism.

ANAEROBIC BIOCORROSION

$$(\text{CORROSION PRODUCTS}) + \text{ACID} \xrightarrow{\text{anoxic}} H_2S \uparrow + PH_3 \uparrow$$

fast   slow
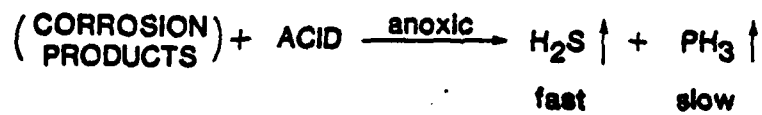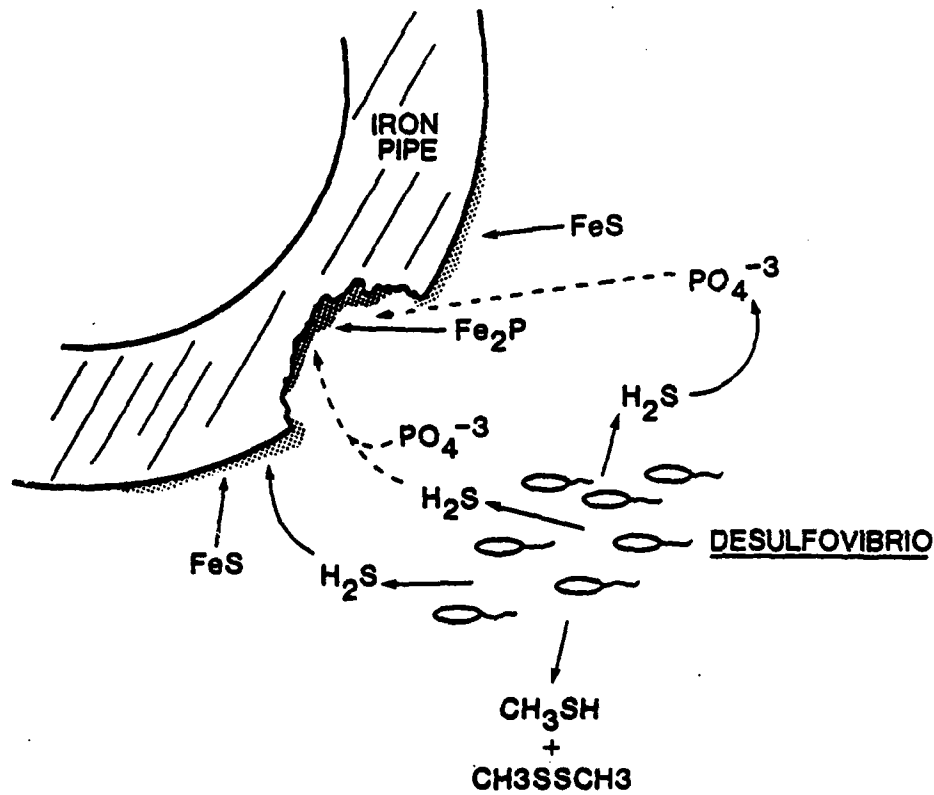
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4. TITLE AND SUBTITLE

The Mechanism of Anaerobic (Mircrobial) Corrosion

5. AUTHOR(S)

W. P. Iverson and G. J. Olson

11. ABSTRACT (A 200-word or less factual summary of most significant information. If document includes a significant bibliography or literature survey, mention it here)

This report in the form of three papers describes research into the role of bacteria in anaerobic corrosion processes. During the year we have given more evidence for a novel mechanism of anerobic corrosion in which a volatile, highly reactive phosphorous compound is produced as a result of the activities of sulfate-reducing bacteria (<u>Desulfovibrio</u> <u>desulfuricans</u>).

12. KEY WORDS (Six to twelve entries; alphabetical order; capitalize only proper names; and separate key words by semicolons)

Anaerobic; bacterial; corrosion; <u>Desulfovibrio</u>; hydrogen sulfide; hypophosphite reduction; iron phosphide; mechanism; phosphine; sulfate-reducing bacteria; volatile phosphorus component.

END

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